resolution, preliminary evidence for the presence of both non-haem and haem iron and flavin has been obtained. The present system is unusual in that the spectral properties of the active preparation resemble microsomal cytochrome *P*-420 (Ohmura & Sato, 1964) rather than the *P*-450 type observed by Katagiri *et al.* (1968). Crude extracts that have not been exposed to ethanol show the dithionite-carbon monoxide-treated peak at 417–418nm. Although ethanol is known to convert microsomal cytochrome *P*-450 into *P*-420 (Ichikawa, Yamano & Fujishima, 1969), this in other systems is accompanied by a loss of enzyme activity not observed in the present system.

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Enzymic Reduction of the C-Terminal Dehydroalanyl-lysine Sequence in Nisin

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Inactivation of the polypeptide antibiotics nisin and subtilin by a non-proteolytic enzyme from several species of *Bacillus* has been reported, but no significant molecular changes were observed in the enzyme-inactivated nisin (Jarvis, 1967a,b). Gross, Morrell & Craig (1969) have shown that both nisin and subtilin contain the C-terminal sequence dehydroalanyl-lysine; loss of biological activity results from the hydrolytic cleavage of this terminal sequence in nisin (Gross & Morrell, 1967). It seemed possible, therefore, that the 'nisinase' might act as a dehydroalanine reductase or as a dehydropeptidase.

Purified nisin (25mg) was incubated for 72h at 30°C in phosphate buffer (0.1M, pH 6.8) containing 100 μ M-Co²⁺ and -Ca²⁺, with an enzyme preparation from *Bacillus cereus* (B. Jarvis, unpublished work). Controls containing heat-inactivated enzyme were incubated concurrently. After incubation the mixtures were acidified to pH 1 and heated for 5min at 100°C. Bioassay showed a net inactivation of 76% in the test

compared with the controls. After partial hydrolysis (110°C for 10min; M-HCl in acetic acid) (Gross & Morrell, 1967) the elution patterns of the nisin preparations from Sephadex G-25 (80cm $\times$ 2.5cm; 0.2M-acetic acid) showed no differences. However, t.l.c. of the pyruvyl-lysine peak (derived from dehydroalanyl-lysine) revealed the presence of alanyl-lysine in the eluate from the enzyme-inactivated nisin.

Semiquantitative high-voltage electrophoresis of acid hydrolysates of the nisin preparations treated for 10 days at room temperature with an excess of methylmercaptoacetate under N₂ (Gross & Morrell, 1968) showed a decrease in the carboxymethylcysteine content of the enzyme-inactivated nisin relative to the controls. Treatment of the nisin preparations (1.5nmol) with 2.5 μ Ci of L-[¹⁴C]cysteine (18.3 μ Ci/ μ mol) at pH 7.0 (0.1M-phosphate buffer) and 30°C for 24h showed a 23% decrease in the binding of cysteine to the inactivated nisin (13950 d.p.m.) compared with the controls (18040 d.p.m.). The theoretical decrease in binding is 26%, assuming that the 76% inactivation results from the reduction of only one of the three $\alpha\beta$ -unsaturated amino acid residues/mol of nisin. The [¹⁴C]cysteine-labelled nisins, mixed with 5mg of carrier nisin, were subjected to chromatography on Sephadex G-25 after partial hydrolysis (see above) and the eluate was monitored for extinction and radioactivity. Considerably less radioactivity was associated with the 'pyruvyl-lysine' peak in the inactive nisin than in the controls, but similar amounts of radioactivity were observed in the de-(dehydroalanyl-lysine)-nisin peaks. These results are consistent with the hypothesis of enzymic reduction of the C-terminal dehydroalanyl-lysine sequence to alanyl-lysine.

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